

4R AS: 5'-TTC TCG TCG ACG TCT TCT TGG TTC AAT GGC ATT GTT (SEQ. ID
No. 4)

**Oligonucleotides used for the generation of FVIII compatible ends to clone in FVIII
intron 13 location**

-Generation of I13A

5' Bgl II: 5'-AAT ATG GAG AGA GAT CTA GCT TCA GG (SEQ. ID No. 3)

FVIII 13A-AS: 5'-A **AT GCA T** (AA ACA AAC) CTG GGT TTT CCA TCG ACA TGA A
(SEQ. ID No. 21) **Nsi I site** FIX splice donor

-Generation of I13C

FVIII 13 C-S: 5'- A **AC GCG T** (AT TCT TTT ACA TTT CAG) GTC TAT GGA TTC TGG
GGT (SEQ. ID No. 22) **Mlu I site** FIX splice acceptor

4R AS: 5'-TTC TCG TCG ACG TCT TCT TGG TTC AAT GGC ATT GTT (SEQ. ID
No. 4)

REMARKS

Applicants submit this Amendment in response to the Notice to Comply with
Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino
Acid Sequence Disclosures mailed March 5, 2003. A copy of the Notice is enclosed.

In order to comply with the requirements of 37 C.F.R. 1.821-1.825, Applicants wish to substitute this amended Sequence Listing for the sequence listing submitted on July 23, 2001. SEQ. ID No. 23 and SEQ. ID No. 24 did not appear on the Sequence Listing for the parent application, yet the sequences themselves were disclosed within the drawings. Accordingly, this substitute Sequence Listing contains no new matter.

Applicants have further amended the specification to identify each sequence on the Sequence Listing within the text of the specification. Because the sequences were within a drawing, applicants have amended the Brief Description of the Drawings in order to incorporate the new sequence identification numbers. SEQ. ID No. 13 has been removed from the specification and from the substitute Sequence Listing pursuant to 37 C.F.R. 1.822 (4)(e) and 1.821(a) because it contains an undefined break in the sequence disclosure, and the peptide fragments flanking the undefined region are too short for inclusion. Finally, Annex 2 has been added to the specification. Annex 2 appeared in its entirety in the parent application, Application No. 09/526,935. Accordingly, no new matter has been added by this amendment.

Attorney Docket No.: **6478.1441-01**

Application No.: 09/880,887

Filing Date: June 15, 2001

If there is any fee due in connection with the filing of this Statement, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: April 3, 2003

By: 

Sanya Sukduang
Reg. No. 46,390

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

APPENDIX

In the Specification:

Please amend the specification as follows:

In the Amendment filed December 23, 2002, please amend the description of FIG. 1. on page 2, lines 11-12, as follows:

FIG. 1. Schematic view of the Factor IX intron I inserted in the Factor VIII intron 1 location [.] , **where SEQ. ID No. 23 defines the 5' end, and SEQ. ID No. 24 defines the 3' end.**

Please amend page 4, lines 1-13, of the specification as follows:

Four arginines replace according to the invention the B-domain of the FVIII protein. They are introduced by the oligonucleotides used for the cloning of the two fragments surrounding the B-domain (see Fragments 2 and 3 of Table 1), namely the oligonucleotides 4R AS (SEQ. ID No. 4) and 4R S (SEQ. ID No. 5). The Sal I site was generated by the coding sequence of the arginines as follows:

SAL I SITE

4R S :5'-A AGA CGT CGA CGA GAA ATA ACT CGT ACT ACT CTT (SEQ. ID No. 5)

4R AS TTG TTA CGG TAA CTT GGT TCT GCA GCT GCT CTT (SEQ. ID No. 4)

CORRESPONDING PEPTIDIC SEQUENCE:

Pro Arg Arg Arg Arg Glu Ile Thr Arg Thr Thr Leu (SEQ. ID No. 12)

In the wild-type FVIII the peptidic sequence is:

Pro-Arg-Domain B-Arg-Glu [(SEQ ID No. 13)]

Please amend page 6, lines 1 through 5, of the specification as follows:

The FIX T11 sequence (SEQ. ID No. 9) used according to the invention in different locations of the FVIII cDNA starts after the coding sequence by the splice donor sequence and ends by the splice acceptor sequence of the truncated intron 1. The upper case letters start after and stop before the Nsi I and Mlu I restriction sites, respectively. **For details, see Annex 2.**

Please add Annex 2 to the specification, following the sequence listing:

Annex 2

Oligonucleotides used for introducing TFIX11 in FVIII sequence

The oligonucleotide sense is always presented first.

Oligonucleotides used for introducing two restriction sites in TFIX11:

FVIII IB-S: 5'- C AT GCA T CC TTT TTT AAA ATA CAT TGA G (SEQ. ID No. 14)

Nsi I site

FVIIB-AS: 5'- A AC GCG T TA ATT CTT TAG TTT TAG CA (SEQ. ID No. 15)

Mlu I site

Oligonucleotides used for the generation of FVIII compatible ends to clone in

FVIII intron 1 location

-Generation of I1 A

FVIII ATG: 5'-ACA CCC ATG GAA ATA GAG CTC TCC ACC TGC (SEQ. ID No. 1)

FVIII IA-AS: 5'-A AT GCA T (AA ACA AAC) CTT GCG TCC ACA GGC AGC TC (SEQ.

ID No. 16) Nsi I site FIX splice donor

-Generation of I1 B

FVIII IC-S: 5'-A AC GCG T (AT TCT TTT ACA TTT CAG) ATT TCC TCC TAG AGT

GCC (SEQ. ID No. 17) Mlu I site FIX splice donor

AAA ATCT

FVIII 585-AS: 5'-TTC TCT ACA T AC TAG T AG GGC (SEQ. ID No. 18)

endogenous FVII Spel site

Oligonucleotides used for the generation of FVIII compatible ends to clone in FVIII

intron 12 location

-Generation of I12A

5' Bgl II: 5'-AAT ATG GAG AGA GAT CTA GCT TCA GG (SEQ. ID No. 3)

FVIII 12-AS: 5'-A **AT GCA T** (AA ACA AAC) TGT GCA TGA TGT TGG AGG CT (SEQ.

ID No. 19) **Nsi I site** FIX splice donor

-Generation of I12C

FVIII 12C-S: 5'-A **AC GCG T** (AT TCT TTT ACA TTT CAG) GCA TCA ATG GCT ATG

TTT (SEQ. ID No. 20) **Mlu I site** FIX splice acceptor sequence

4R AS: 5'-TTC TCG TCG ACG TCT TCT TGG TTC AAT GGC ATT GTT (SEQ. ID

No. 4)

Oligonucleotides used for the generation of FVIII compatible ends to clone in FVIII

intron 13 location

-Generation of I13A

5' Bgl II: 5'-AAT ATG GAG AGA GAT CTA GCT TCA GG (SEQ. ID No. 3)

FVIII 13A-AS: 5'-A **AT GCA T** (AA ACA AAC) CTG GGT TTT CCA TCG ACA TGA A

(SEQ. ID No. 21) **Nsi I site** FIX splice donor

Attorney Docket No.: **6478.1441-01**
Application No.: 09/880,887
Filing Date: June 15, 2001

-Generation of I13C

FVIII 13 C-S: 5'- A **AC GCG T** (AT TCT TTT ACA TTT CAG) GTC TAT GGA TTC TGG

GGT (SEQ. ID No. 22) **Mlu I site** FIX splice acceptor

4R AS: 5'-TTC TCG TCG ACG TCT TCT TGG TTC AAT GGC ATT GTT (SEQ. ID

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FINNEGAN
ENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com